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(54) Title: VACCINE COMPOSITIONS

(57) Abstract

The invention relates to a vaccine composition adapted for mucosal administration, and in particular for intranasal administration, the composition comprising an influenza virus antigen or antigens, such as haemagglutinin and neuraminidase influenza virus antigens, and an effective adjuvant amount of a chitosan. The compositions can be used to immunise a host against infection with influenza, the chitosan serving to enhance the stimulation of a protective IgA mucosal immune response and an IgG systemic immune response.

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VACCINE COMPOSITIONS

The invention relates to a vaccine composition for intranasal administration comprising influenza virus antigens and a mucosal adjuvant. The invention also relates to a method of immunising a patient against influenza by administering the said composition to the patient, and a method of enhancing the immunogenicity of an influenza viral antigen when administered intranasally, by co-administering therewith the said adjuvant. In a further aspect, the invention provides the use of an influenza viral antigen in combination with a chitosan for the manufacture of a vaccine composition for intranasal administration to immunise a patient against influenza.

Current influenza vaccines consist of either inactivated whole virus, disrupted virus (split vaccines) or purified preparations of the membrane glycoproteins haemagglutinin (HA) and neuraminidase (NA) sub-unit vaccines. Haemagglutinin and neuraminidase are the antigens to which protective antibody responses are directed, haemagglutinin being the major protective antigen. Estimates of the efficacy of these parenterally administered vaccines vary greatly. Such vaccines are

believed to act primarily by eliciting circulating anti-haemagglutinin IgG antibodies that transudate into the lower respiratory tract.

M.L. Clements *et al*, J. Clinical Microbiology 24, 157-160, 1986, have previously reported that both secretory IgA and serum IgG participate in immunity to influenza virus. Moreover, in mice, a number of published studies have demonstrated the importance of respiratory IgA to protection against influenza infection. It has also been found that an advantage of stimulating a local IgA response to influenza is that it is often of a broader specificity than the serum response and thus can provide cross-protection against viruses possessing haemagglutinin molecules different from those present in the vaccine. Accordingly, influenza vaccines that elicit both local secretory and serum anti-haemagglutinin responses should provide superior immunity to current vaccines. However, parenteral vaccination (intramuscular, sub-cutaneous etc) is not effective at eliciting local antibody production, if there has been no previous mucosal exposure (e.g. infection). In order to stimulate the mucosal immune system, the vaccine must be applied topically to a mucosal surface.

Mucosal administration of influenza vaccine would have a number of advantages over traditional parenteral immunisation regimes. Paramount amongst these are more

effective stimulation of the local mucosal immune system of the respiratory tract and the likelihood that vaccine uptake rates would be increased because the fear and discomfort associated with injections would be avoided. Accordingly, a number of attempts have been made to develop mucosal influenza vaccines. A drawback however is that inactivated vaccines are often poorly immunogenic when given mucosally. In order to overcome this problem, different approaches to improving the immunogenicity of flu vaccines given orally or intranasally have included the use of the B sub-unit of cholera toxin (CTB) as an adjuvant, encapsulation of the vaccine in a variety of microspheres, and the use of live attenuated strains. To date however no practical means of enhancing the immunogenicity of mucosally administered flu vaccines has been developed.

It has now been found by the Applicants that by administering the haemagglutinin and neuraminidase antigens of influenza together with a particular chitosan derivative in an intranasal formulation, it is possible to achieve good IgG and good IgA responses.

Chitosans are derivatives of chitin or poly-N-acetyl-D-glucosamine in which the greater proportion of the N-acetyl groups have been removed through hydrolysis.

Chitosans have previously been used in pharmaceutical formulations and are disclosed in EP-A-0460020 as mucosal

absorption enhancers, However, EP-A-0460020 does not disclose or suggest that the chitosan could provide an adjuvant effect when administered in a vaccine composition.

The present Applicants have now found that if a chitosan is incorporated into intranasal vaccine compositions containing the neuraminidase and haemagglutinin antigens of influenza virus, good systemic and local immune responses are produced.

Accordingly, in a first aspect the invention provides a vaccine composition adapted for mucosal administration; the composition comprising an influenza virus antigen(s); and an effective adjuvant amount of chitosan.

The vaccine composition is preferably adapted for intra nasal administration.

Preferably the composition contains both haemagglutinin and neuraminidase influenza virus antigens.

In a preferred embodiment the invention provides a vaccine composition adapted for intranasal administration; the composition comprising purified haemagglutinin and neuraminidase influenza virus antigens; and an effective adjuvant amount of a chitosan.

It is preferred that the purified haemagglutinin and

neuraminidase antigens are present in the form of rosettes. The rosettes preferably are particles with a radius in the range 10 to 25 nanometres.

It is preferred that the rosettes are substantially free of lipid and, moreover, it is preferred that the purified haemagglutinin and neuraminidase antigens preparation as a whole is substantially free of lipids.

An example of a haemagglutinin/neuraminidase preparation suitable for use in the compositions of the present invention is the "Fluvirin" product manufactured and sold by Evans Medical Limited of Speke, Merseyside, United Kingdom, and see also S. Renfrey and A. Watts, Vaccine, 1994, Volume 12, Number 8, pp 747-752.

The compositions can contain influenza virus antigens from a single viral strain, or from a plurality of strains. For example, the composition can contain antigens taken from up to three or more viral strains. Purely by way of example the composition can contain antigens from one or more strains of influenza A together with antigens from one or more strains of influenza B.

Preferably the chitosan is water-soluble.

The chitosan may advantageously be a deacetylated chitin which is at least 80% deacetylated.

Preferably the chitosan is at least 85% de-acetylated, and more preferably is 88% to 90% de-acetylated.

A particular de-acetylated chitosan is the "Sea Cure +" chitosan glutamate available from Protan Biopolymer A/S, Drammen, Norway.

In a further aspect, the invention provides a method of immunising a host against infection with influenza, which method comprises administering to a mucosal surface of the host (preferably intranasally) a vaccine composition comprising influenza virus antigens such as purified haemagglutinin and neuraminidase antigens together with an effective adjuvant amount of a chitosan as hereinbefore defined.

In a further aspect, the invention provides a method of enhancing a protective IgA mucosal immune response and an IgG systemic immune response by administering (preferably intranasally) to a mucosal surface of the patient a vaccine composition comprising influenza virus antigens such as purified haemagglutinin and neuraminidase; and an effective adjuvant amount of a chitosan as hereinbefore defined.

In a still further aspect, the invention provides a method of enhancing the immune response of influenza virus antigens such as purified haemagglutinin and neuraminidase,

(e.g. when administered intranasally), by co-administering therewith a chitosan as hereinbefore defined.

The compositions of the invention, and in particular intranasal compositions, can be formulated as liquids or dry powders, for administration as aerosols or drops.

Compositions for administration as nasal drops may contain one or more excipients of the type usually included in such compositions, for example preservatives, viscosity adjusting agents, tonicity adjusting agents, buffering agents and the like.

In order to ensure that the chitosan remains soluble in the aqueous medium, and to ensure also that the haemagglutinin is not adversely affected by too acidic a pH, a solution (e.g. for intranasal administration) preferably has a pH in the range 5.5 to 6.5, most preferably approximately pH6.

The present invention also contemplates the provision of means for dispensing intranasal formulations of influenza virus antigens such as purified surface antigen, and chitosan. A dispensing device may, for example, take the form of an aerosol delivery system, and may be arranged to dispense only a single dose, or a multiplicity of doses.

The vaccine will be administered to the patient in an

amount effective to stimulate a protective immune response in the patient. For example, the vaccine may be administered to humans in one or more doses, each dose containing 1-250 microgrammes and more preferably 5-50 microgrammes of protein prepared from each virus strain. For example, where haemagglutinin and neuraminidase preparations are prepared from three virus strains, e.g. 2 x Influenza A and 1 x Influenza B, a total dose of viral protein administered could be in the range 15-150 microgrammes.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the serum IgG anti-haemagglutinin response in mice immunised with PSA. Each bar represents the geometric mean titre of four mice. The error bars represent 1 standard error of the mean. The cut-off value is 50 which is the lower limit of detection.

Figure 2 illustrates the nasal IgA anti-haemagglutinin response in mice immunised with purified surface antigen (PSA). As with Figure 1, each bar represents the geometric mean titre of four mice, and the error bars represent 1 standard error of mean.

Figures 3a and 3b illustrate the determination of nasal and pulmonary anti-haemagglutinin secreting cells of mice immunised with purified surface antigen, using

ELISPOT. Figure 3a uses a log scale whilst Figure 3b uses a linear scale.

EXAMPLE 1

Preparation of influenza B purified surface antigen/chitosan glutamate formulation

1A. A solution of 1% chitosan glutamate, a medium viscosity de-acetylated chitin having approximately 11% residual N-acetyl groups, was prepared by dissolving the chitosan glutamate in 0.8% sodium chloride. The grade of chitosan glutamate used was "Sea Cure + 210", available from Protan Biopolymer A/S, Drammen, Norway.

1B. Influenza purified surface antigen (PSA) containing both Influenza A and Influenza B protein, commercially available from Evans Medical Limited, Speke, Merseyside, United Kingdom, under the Trade Mark "Fluvirin", was made up in phosphate buffered saline to give a protein concentration of approximately 1mg/ml. The PSA consists almost entirely of the spike protein haemagglutinin (HA), although it does contain some neuraminidase.

1C. A 1:1 mixture of the chitosan glutamate solution and the PSA solution was prepared to give an intranasal vaccine composition containing 0.5% chitosan glutamate (11% acetylated), 0.8% NaCl, 0.1% PSA and phosphate buffer to

give a solution pH of 6.

1D. Control solutions containing the same concentrations of PSA but not chitosan glutamate, and the same concentrations of chitosan glutamate but no PSA, were also prepared. In addition, a composition comprising the same concentration of PSA adsorbed on to the known adjuvant alhydrogel (aluminium hydroxide) was prepared. The PSA was adsorbed on to the alhydrogel overnight at 4°C.

EXAMPLE 2

Mice Immunisation Studies

2A. The four compositions prepared as described in Example 1 were administered to groups of twelve adult (6-8 weeks) female BALB/c mice as follows:

Group 1. 20µl (10µl per nostril) PSA/chitosan solution administered intranasally. PSA dose = 10µg.

Group 2. 20µl PSA administered intranasally (total PSA dose = 10µg).

Group 3. 200µl PSA/alhydrogel administered subcutaneously (PSA dose = 10µg).

Group 4. 20µl chitosan solution administered intranasally.

Group 5. 20µl PSA (10µl per nostril) administered daily for three days. (Groups of four mice employed for this study).

2B. The immunisation procedure was carried out three times

at monthly intervals, with the exception of Group 5 where the mice were immunised with three successive daily doses. The immunisation and sampling regime is shown in Table 1.

Immunisation and sampling regime

TABLE 1

Immunisation	Day	Sample	Day
1	1	1	21
2	30	2	44
3	57	3	71+72

At each sampling point four mice from each group were terminally bled by cardiac puncture, their heads were removed and their nasal passages lavaged with 1ml PBS + 1% bovine serum albumin. Group 5 contained four mice only so blood was obtained by tail puncture for the first two samples and nasal washes were only performed at the third sampling point.

Antibody assays

In all assays whole influenza vaccine (WIV) was used as antigen. Although WIV is only ~50% HA the assays were thought to be measuring primarily anti-HA antibodies. This assumption was confirmed by substituting PSA (~100% HA) for WIV and repeating some assays. The results were similar

with either antigen. HA-specific serum IgG and nasal IgA antibodies were measured by Enzyme Linked Immunosorbant Assay (ELISA). After correcting for background, the individual optical density (OD) dilution curves were plotted and the titre values determined. The titre was determined as the dilution of serum that gave an OD reading of 0.2 or the dilution of nasal wash that gave an OD reading of 0.1.

As well as taking nasal washes at the third sample lymphocytes were isolated from the mucous membranes of the nasal cavity and the lungs and the local immune response analysed by ELISPOT.

Results

1. **Serum anti-HA serum response**

Purified Surface Antigen (Figure 1 and Table 2)

As expected a good serum response was elicited by subcutaneous (S\C) immunisation with PSA + Alhydrogel. All the animals tested had seroconverted after the primary immunisation and the geometric mean titre (GMT) was good. The response increased after each boost, the GMT after the third dose was very high (~800,000). In contrast the serum response to PSA alone administered intranasally was poor: only two of four mice had seroconverted after the first

dose, none of the mice tested had serum HA antibodies after the second dose (these are separate mice from those tested after the first immunisation) and although all animals tested had seroconverted after the third dose the GMT was lower than that of animals receiving one dose of PSA + Alhydrogel. Chitosan enhanced the serum response of intranasally administered PSA; after the third vaccination the antibody response in mice that received PSA + chitosan was 360-fold greater than that of mice receiving PSA alone I\N. The magnitude of the serum response in the PSA + chitosan mice was very similar to that of S\C immunised mice; in fact there was no statistical difference in the GMT's of the two groups at any sampling point (Students t-Test $p>0.01$).

Some mice were immunised three times on successive days with PSA alone administered intranasally to study whether this regime had advantages over the once monthly regime. Although all the mice in this group had detectable serum antibodies 21 days after the first dose and the GMT at this time point was greater than in mice that had received a single dose of PSA intranasally, the number of mice seropositive decreased during the course of the study although the GMT did not (in this group the same mice were sampled at each time point). At the final time point the GMT of the mice on the monthly regime was an order of magnitude greater than mice on the daily regime.

TABLE 2

Serum IgG anti-HA response in PSA immunised mice

Group	Post-Dose 1 Seroconversion	GMT	Post-Dose 2 Seroconversion	GMT	Post-Dose 3 Seroconversion	GMT
PSA + Chitosan	4/4	557	4/4	40504	4/4	653113
PSA I/N	2/4	67	0/4	<50	4/4	1818
PSA S/C	4/4	2339	4/4	35196	4/4	816552
PSA 3 daily doses	4/4	182	3/4	229	2/4	180

[a] No. positive/No. tested

[b] Geometric Mean Titre

2. Nasal wash IgA anti-HA response

Purified Surface Antigen (Figure 2 and Table 3)

PSA + Alhydrogel given subcutaneously was very poor at inducing a nasal IgA response which is consistent with our previous findings and those of others. PSA alone given intranasally was also a poor mucosal immunogen although it was slightly better than subcutaneous immunisation in terms of the number of animals responding. Adding chitosan greatly boosted the IgA response, although the response was low after the first dose, HA-specific IgA could be detected in three out of four mice. The IgA response was boosted greatly in these mice by the second immunisation. The final immunisation had little effect; in fact the mean specific IgA levels had decreased slightly.

TABLE 3

Nasal IgA anti-HA response in PSA immunised mice

Group	Post-Dose 1		Post-Dose 2		Post-Dose 3	
	Mucosal-conversion	GMT	Mucosal-conversion	GMT	Mucosal-conversion	GMT
PSA + Chitosan	3/4	2.26	4/4	282.81	4/4	184.47
PSA I/N	0/4	<1	1/4	1.20	3/4	2.31
PSA S/C	0/4	<1	0/4	<1	2/4	1.32
PSA 3 daily doses					0/4	<1

[a] No. positive\No. tested

[b] Geometric Mean Titre

Responses to Chitosan Alone

The sera and nasal lavage fluid from the control mice immunised with chitosan alone were negative in all the assays.

Local anti-HA antibody secreting cell response (ASC) in nasal and pulmonary tissues

Lymphocytes were isolated from the nasal mucosa and lung parenchyma of groups of four mice at the third sampling point. Lymphocytes from individual mice were pooled and assayed for cells secreting IgA, IgG and IgM anti-flu antibodies using ELISPOT. The results are shown in Figures 3a and 3b.

B cells secreting HA-specific antibodies were detectable in the nasal and lung tissue of all groups. There were far greater number of such cells in the PSA + chitosan group and this is most apparent when the results are plotted on a linear scale (Figure 3b). In all cases, except subcutaneously immunised mice, IgA antibody secreting cells (ASC) predominated in the nasal cavity whereas either IgG or IgM predominated in the lungs. The magnitude of the response is similar in the lungs and nose of PSA + chitosan mice.

The aforementioned examples are merely exemplary of the present invention and are not intended in any way to limit the scope of the invention which is defined solely by the Claims appended hereto.

CLAIMS

1. A vaccine composition adapted for mucosal administration; the composition comprising an influenza virus antigen; and an effective adjuvant amount of chitosan.

2. A vaccine composition according to Claim 1 which is adapted for intranasal administration.

3. A vaccine composition according to Claim 1 or Claim 2 which contains both haemagglutinin and neuraminidase influenza virus antigens.

4. A vaccine composition according to Claim 1 which is adapted for intranasal administration; the composition comprising purified haemagglutinin and neuraminidase influenza virus antigens; and an effective adjuvant amount of chitosan.

5. A vaccine composition according to Claim 3 or Claim 4 wherein the haemagglutinin and neuraminidase influenza are present in the form of rosettes having a radius in the range 10 to 25 nanometres.

6. A vaccine composition according to any one of the preceding Claims wherein the chitosan is a deacetylated chitin which is at least 80% deacetylated.

7. A vaccine composition according to Claim 6 wherein the chitosan is at least 85% deacetylated.

8. A vaccine composition according to Claim 7 wherein the chitosan is 88% to 90% deacetylated.

9. A vaccine composition according to any one of the preceding Claims wherein the chitosan is water-soluble.

10. A vaccine composition according to any one of the preceding Claims wherein the composition has a pH in the range 5.5 to 6.5.

11. A vaccine composition according to Claim 10 wherein the pH is approximately pH6.

12. A pharmaceutical product comprising a dispensing device adapted to deliver a composition intranasally, in combination with a vaccine composition as defined in any one of the preceding Claims.

13. A pharmaceutical product according to Claim 12 wherein the dispensing device is an aerosol delivery system.

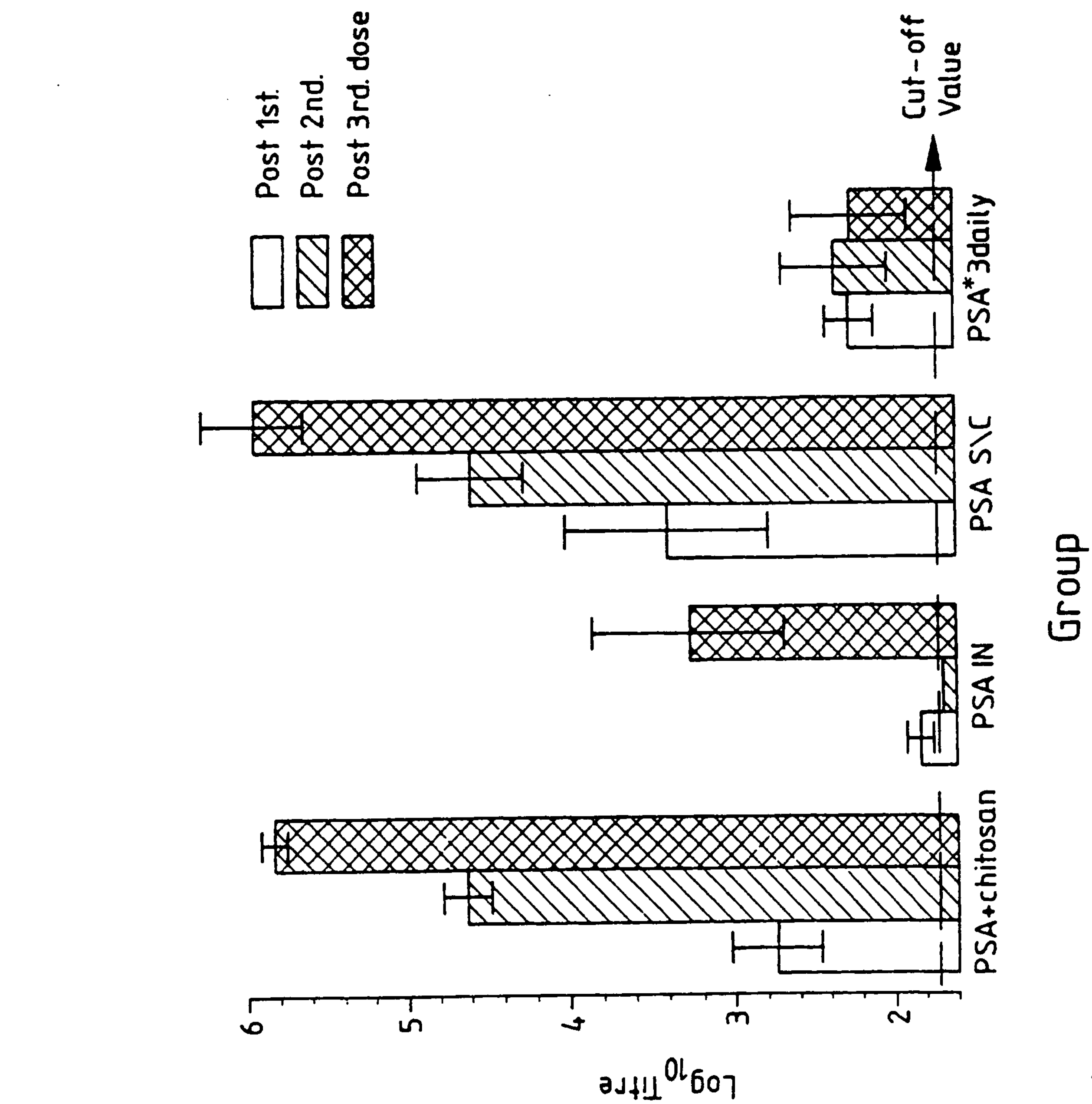
14. A method of immunising a host against infection with influenza, which method comprises administering to a mucosal surface of the host, a vaccine composition

comprising an influenza virus antigen, such as a mixture of purified haemagglutinin and neuraminidase antigens, together with an effective adjuvant amount of a chitosan, as defined in any one of Claims 1 to 11.

15. A method of enhancing a protective IgA mucosal immune response and an IgG systemic immune response by administering to a mucosal surface of a patient a vaccine composition comprising an influenza virus antigen; such as a mixture of purified haemagglutinin and neuraminidase antigens, and an effective adjuvant amount of a chitosan, as defined in any one of Claims 1 to 11.

16. A method of enhancing the immune response of influenza virus antigens such as haemagglutinin and neuraminidase, when administered intranasally, by co-administering therewith a chitosan as defined in any one of Claims 1 to 11.

17. The use of a chitosan as defined in any one of the preceding Claims for the manufacture of an intranasal adjuvant composition for enhancing the immunogenicity of influenza virus antigens such as purified haemagglutinin and neuraminidase when administered intranasally.


Post 1st.
Post 2nd.
Post 3rd. dose
Cut-off Value
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PSA IN
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PSA*3daily
Group
Log10 Titre
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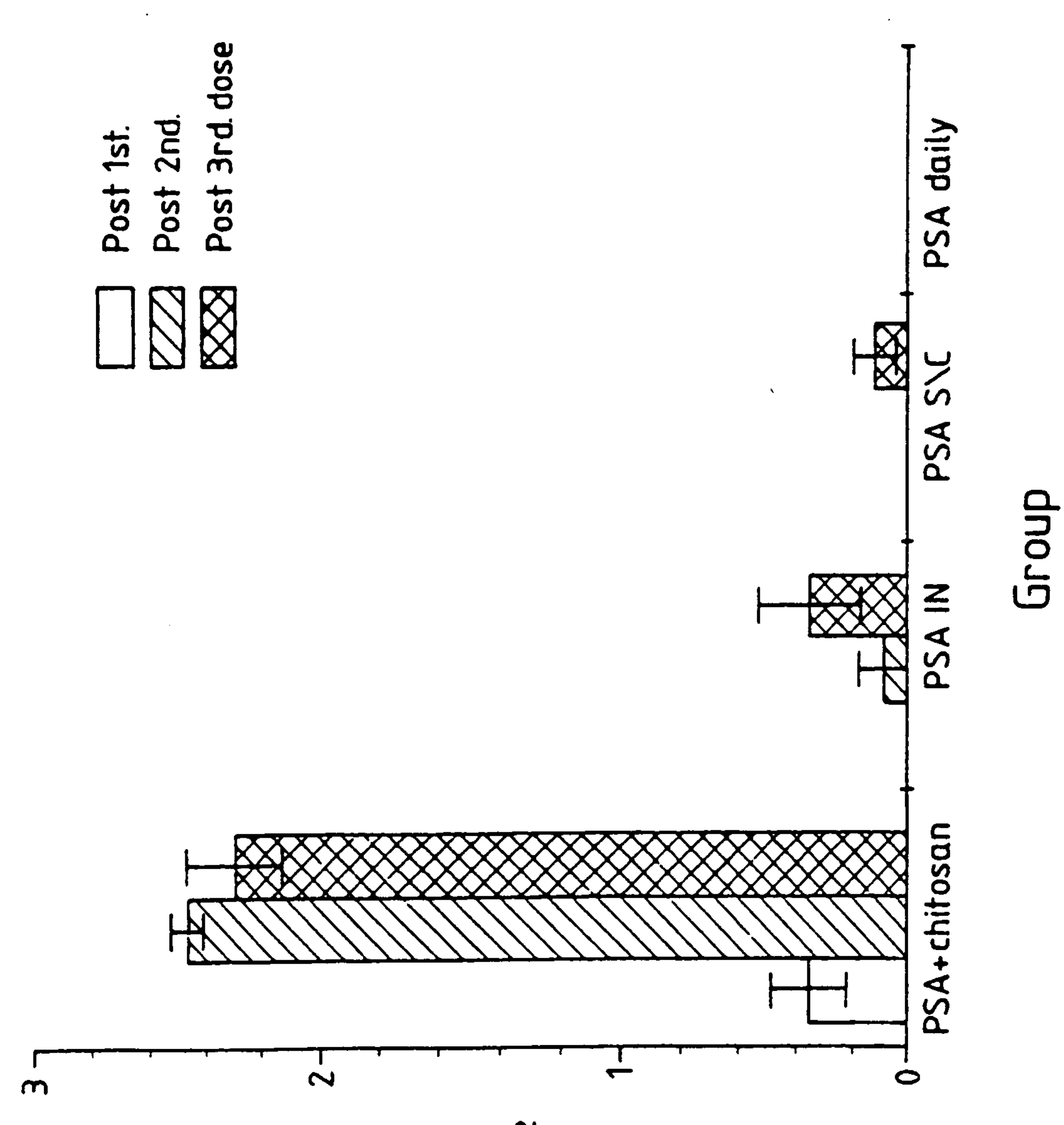
5
4
3
2

Fig.1


Post 1st.
Post 2nd.
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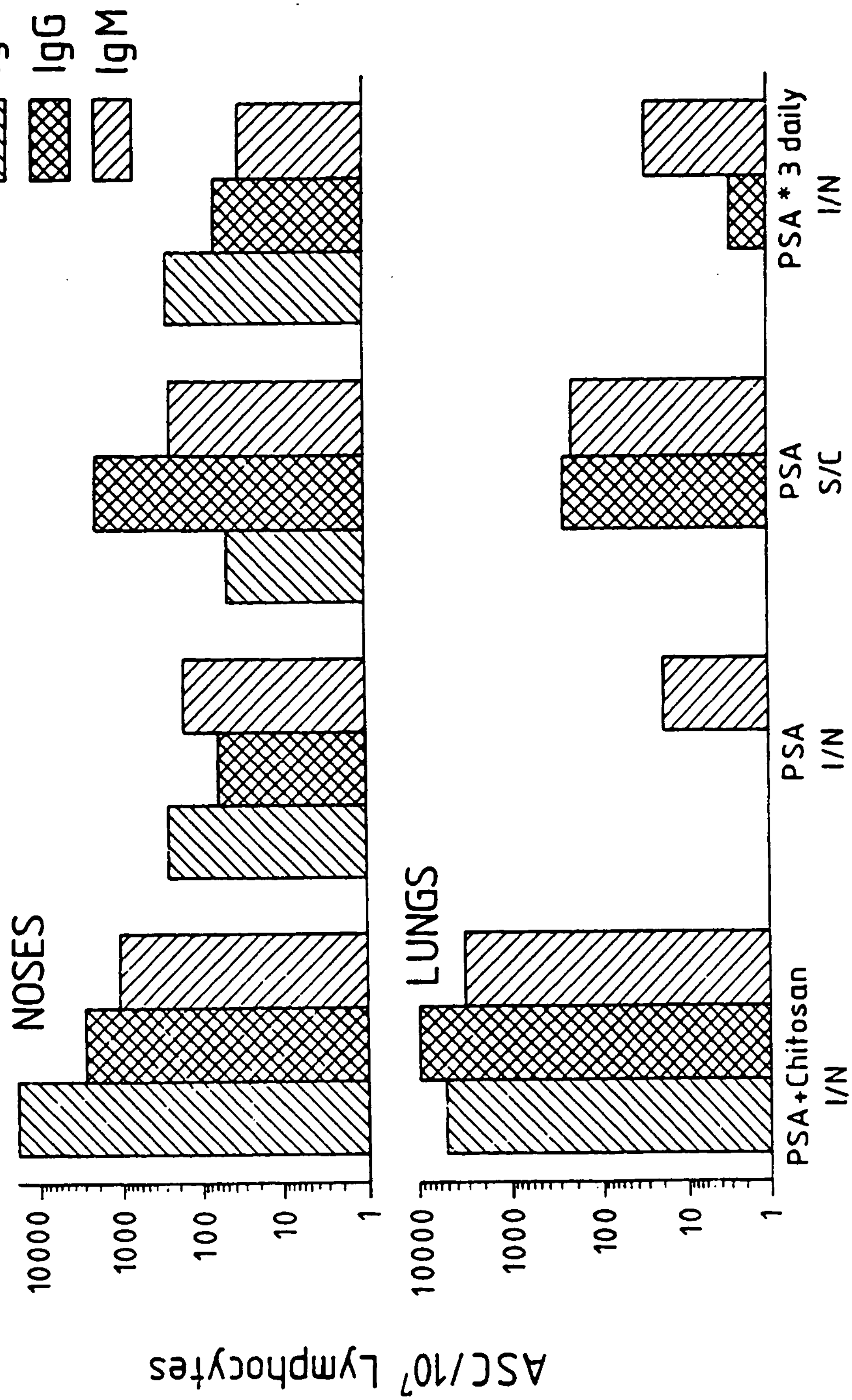
PSA S\C
PSA daily
Group

Fig.2



NOSES
LUNGS
IgA
IgG
IgM
10000
1000
100
10
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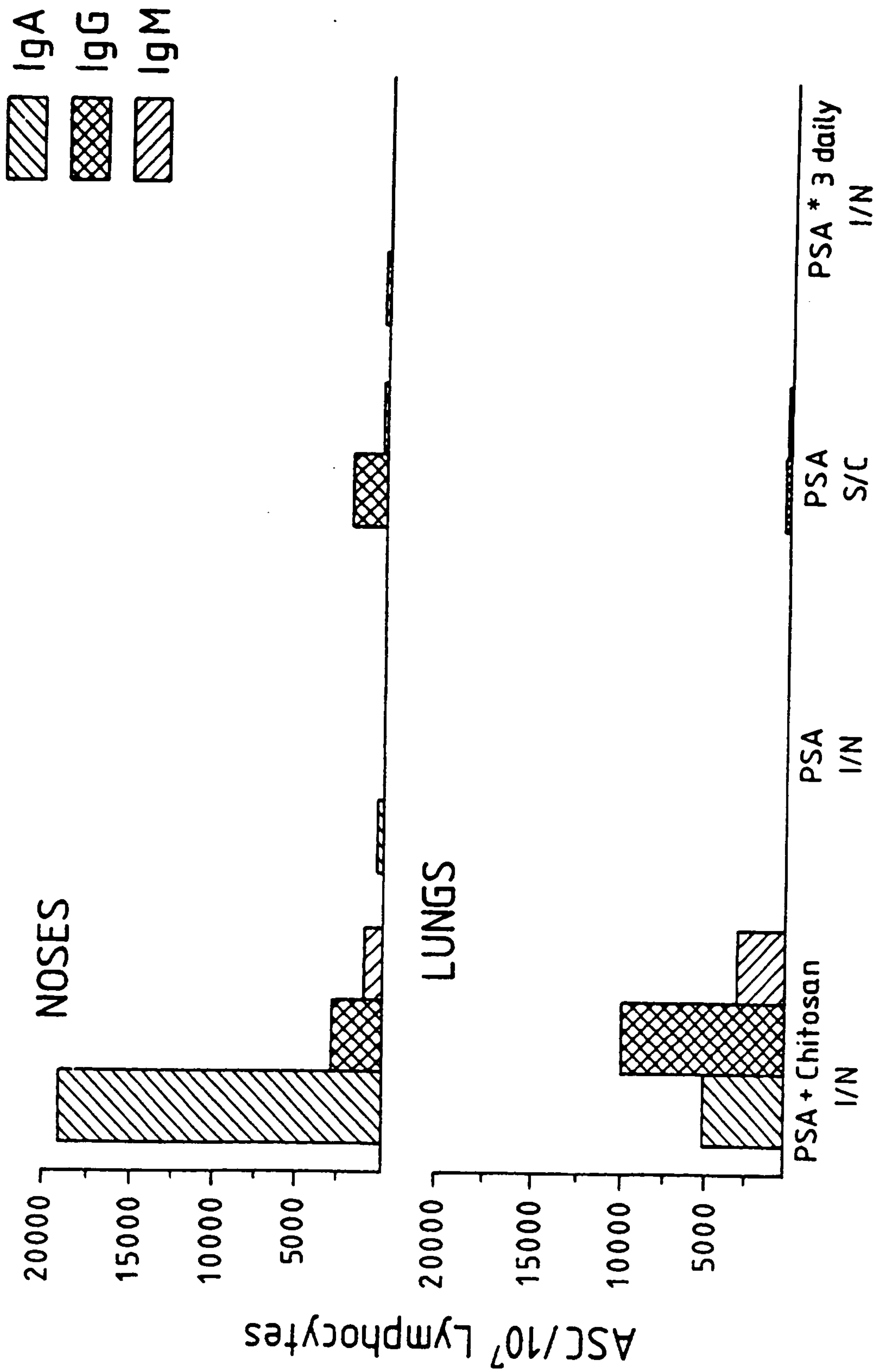
ASC/10^7 Lymphocytes
PSA+Chitosan I/N
PSA I/N
PSA S/C
PSA * 3 daily I/N

Fig.3a



NOSES
LUNGS
IgA
IgG
IgM
20000
15000
10000
5000
ASC/10^7 Lymphocytes
PSA + Chitosan I/N
PSA I/N
PSA S/C
PSA * 3 daily I/N

Fig.3b

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/145 A61K47/36 A61K9/12 A61K39/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE WPI Section Ch, Week 9330 Derwent Publications Ltd., London, GB; Class B04, AN 93-239930 & JP,A,05 163 161 (DENKA SEIKEN KK) , 29 June 1993 see abstract ---	1-17
Y	DATABASE WPI Section Ch, Week 9428 Derwent Publications Ltd., London, GB; Class B04, AN 94-230626 & JP-A-06 166 635 (SUN FIVE KK) , 14 June 1994 see abstract --- -/--	1-17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 1996	20.02.96

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US accession number 93257517; DIALOG SERVER, 1992 INDULEN ET AL 'The antiviral action of a modified bacterial ribonuclease' see abstract & BIOL NAUKI, vol. 4, 1992 pages 87-9, ---	1-17
Y	EP-A-0 506 326 (PROTAN BIOPOLYMER AS & NOBIPOL NOBIPOLS) 30 September 1992 * p.3, l.3-10; p.7, l.48; claim 1 * ---	1-17
Y	US-A-4 659 569 (MITSUHASHI ET AL) 21 April 1987 * col.1, l.42, l.58; claims 1-8 * ---	1-17
A	EP-A-0 183 556 (IHARA CHEM IND CO LTD) 4 June 1986 see claims 1-10 -----	1-17

1

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-506326	30-09-92	US-A-	5169840	08-12-92
		JP-A-	6087751	29-03-94
US-A-4659569	21-04-87	JP-C-	1375994	22-04-87
		JP-A-	57136528	23-08-82
		JP-B-	61040211	08-09-86
		CH-A-	650683	15-08-85
		FR-A,B	2499412	13-08-82
		GB-A,B	2095552	06-10-82
EP-A-183556	04-06-86	JP-B-	6015476	02-03-94
		JP-A-	61268626	28-11-86
		JP-C-	1791018	29-09-93
		JP-B-	4081967	25-12-92
		JP-A-	61130230	18-06-86
		CA-A-	1261264	26-09-89
		JP-B-	7023313	15-03-95
		JP-A-	62123123	04-06-87
		US-A-	4971956	20-11-90